

Fig 1. Interleukin (IL)-12 expression measured by enzyme-linked immunosorbent assay in samples obtained from electroporated tumors pre- and postelectroporation. Each panel represents a single cohort with samples from an individual patient depicted with individual bars. The time and type of biopsy specimen is as described in the x-axis labels and the quantity of IL-12 is depicted in a logarithmic scale on the y-axis. (A) Cohort 1, (B) cohort 2, (C) cohort 3, (D) cohort 4, (E) cohort 5, (F) cohort 6, (G) cohort 7, and (H) mean and standard deviation of IL-12 levels for each cohort. Note that cohort 7 (the maximally administered dose) has six patients whereas all other cohorts have three patients.

out and faded (Fig 3A-3F). The sites of regressed lesions were biopsied at 7 and 18 months (Fig 2D), did not demonstrate evidence of melanoma, and showed only residual pigmentation. In addition, the patient had no evidence of systemic disease by positron emission tomography (PET) or computed tomography (CT) imaging at 20 months post-treatment. Patient 14 (cohort 5) had progressive cutaneous lesions in the right lower extremity (Fig 4A-4B) after multiple surgeries and hyperthermic isolated limb perfusion with melphalan. Six months after the electroporation delivery of pIL-12, the cutaneous lesions started regressing and developing hypopigmentation (halo effect) around them, and this effect persisted and the lesions have regressed further (Fig 4C-4D). A sample pigmented lesion was biopsied and showed only residual melanin pigment without any evidence of tumor. PET imaging, which had previously revealed positive results in the left calf, showed no uptake at 17 months post-treatment and continued to show no evidence of noncutaneous disease. Patient 7 (cohort 3), had an interesting post-treatment

history with a rapidly progressing cutaneous metastases from a primary flank tumor that had been widely resected and irradiated after a local resection. After completing day-39 resection, the patient received dacarbazine therapy. Five months post-electroporation, after having received four cycles of dacarbazine, he had complete regression of all lesions and on a follow-up CT scan had no evidence of disease. At a further follow-up exam, now 24 months after completion of electroporation, he is radiologically and clinically free of disease. Patient 23 (cohort 7) had progressive disease in the thigh and supraclavicular lymph nodes after participating in an autologous tumor vaccine trial. After pIL-12 delivery with electroporation, this patient had partial regression of local thigh lesions as well as regression of a distant supraclavicular lymph node site. In six other patients, uninjected lesions remained stable, with no new lesions developing, during a period of 4 to 20 months after the end of protocol therapy (one from cohort 2, one from cohort 4, two from cohort 6, and two from cohort 7). A statistically significant correlation was

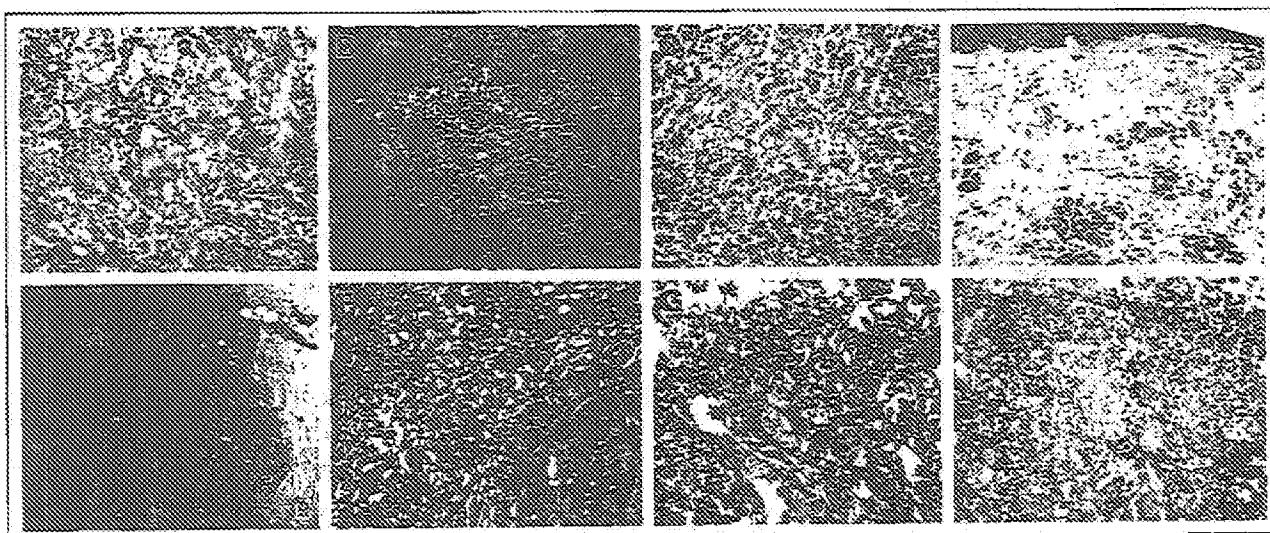


Fig 2. Histologic appearance of electroporated lesions. (A-C) Hematoxylin and eosin-stained tumor samples on patient 9 (cohort 3): (A) Melanoma lesion immediately pre-electroporation (magnification = 200 \times), (B) on day 22 (magnification = 200 \times), (C) on day 39 (magnification = 200 \times), and (D) pigmented nodule with residual melanosis without viable melanoma excised from the chest 18 months after the electroporation procedure was performed (magnification = 200 \times). (E-H) Patient 10 (cohort 4): (E) A 50 \times magnification with hematoxylin and eosin staining with a central viable melanoma tumor surrounded by necrotic tumor removed on day 22. Panel F shows a section from the same tumor at a higher magnification (magnification = 200 \times) showing inflammatory infiltrates. (G, H) Sections from the same patient with CD4 and CD8 immunoperoxidase staining respectively on day 39 (magnification = 200 \times).

lesions demonstrated necrosis (> 20%) at the time of follow-up biopsy or excision performed between 3 and 31 days after the last injection. Because IL-12 has been established to upregulate both adaptive and innate immunity, we also examined lymphocytic infiltrate in

the treated tumors. Electroporated tumors demonstrated CD4⁺CD8⁺ lymphocytic infiltrate in the treated lesions. The experimental regimen was found to be safe and well tolerated, with minimal systemic toxicity and with transient pain associated with the administration of

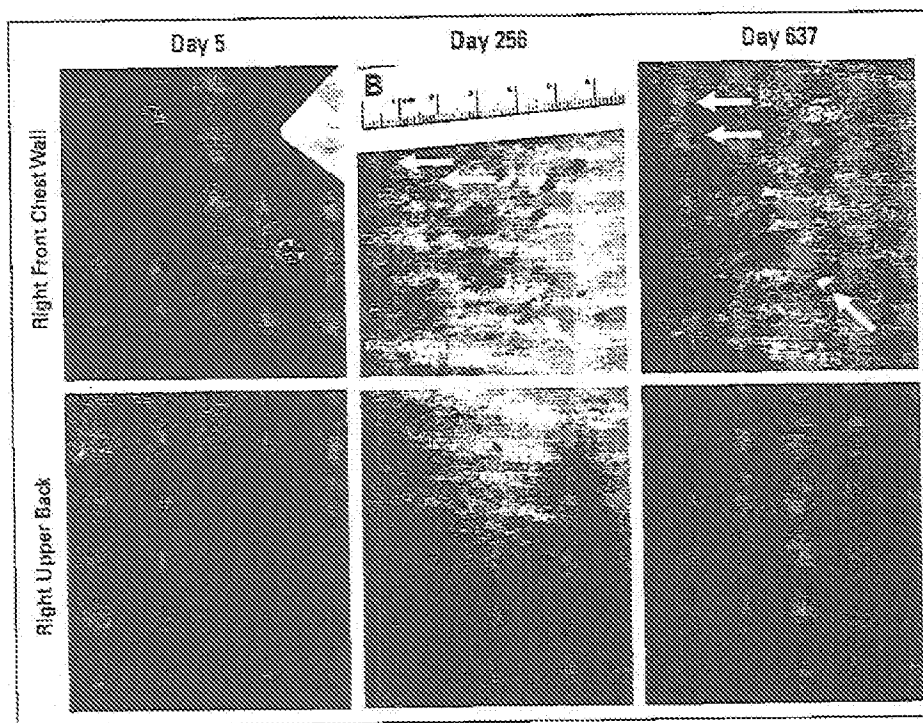


Fig 3. Cutaneous lesions in (A-F) patient 9 from cohort 3 and (G-J) patient 14 from cohort 5. (A-C) Right front chest wall, (D-F) Right upper back. A and D were photographed on day 1 (pretreatment), B and E on day 256, and C and F on day 637. Note that the electroporated lesions (2, 3, 4 in panel A) were resected and the sites are shown by white arrows. The nonelectroporated lesions gradually flatten and fade away (D-F). The seborrheic keratosis (shown by the black arrows) persists whereas the metastatic melanoma lesions flatten and fade with time.

